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A METHOD FOR THE COLLECTION, STORAGE, AND
MONITORING ANALYSIS OF NATURAL SEA WATER

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ADMINISTRATIVE INFORMATION

The work reported was part of a project sponsored by the Atomic Energy Commission, under AT (49-5)2084. The project is described in USNRDL Technical Program Summary for Fiscal Years 1963, 1964, and 1965, 1 November 1962, where it is designated Program A-5, Problem 5.

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ABSTRACT

Natural seawater was required for use in experiments designed to determine the dissolution rate of certain nuclear fuel materials. A literature survey was conducted and a method was devised for the collection, storage and analysis of the water. In brief, the method stipulates: (1) off-shore collection of surface water in polyethylene carboys which are subsequently stored, at a reduced temperature, in a dark location and (2) monitoring biological and chemical composition via microfiltration, chlorinity, oxygen, alkalinity, phosphate and nitrate analyses.

SUMMARY

The purpose of the present report has been to establish a procedural method for the systematic collection, storage and characterization of natural seawater samples. These procedures are considered to be satisfactory for use in dissolution studies which are in progress at this laboratory. They may also be of some value to workers with objectives similar to our own.

INTRODUCTION

During the past few years, considerable effort has been expended to develop nuclear energy systems for space applications. Some of these systems utilize massive amounts of potentially hazardous radio-nuclide fuels. Since space launches from coastal regions are planned, it is necessary to evaluate the radiological hazards that might ensue if a nuclear unit accidentally fell into an ocean area.

A program has been established at USNRDL to determine the rate and extent of radioactivity release from fuels exposed to seawater. The most appropriate liquid medium for such an investigation is an actual ocean environment. However, direct measurement of the activity released under such circumstances is difficult. Consequently it was decided to conduct the studies in the laboratory using a simulated ocean environment.

One choice for such simulation is through the preparation of synthetic seawater. Considerable effort has been devoted to the development of a seawater simulant. However adequate data has been collected^{10,11,13} to show that the corrosive properties of such simulants differ from the corrosive properties of real seawater. Thus, for the intentions of the contemplated radionuclide release investigations, artificial seawater solutions would not be satisfactory. A second choice for laboratory simulation of in situ ocean water consists in solutions taken from the ocean. Although the chemical composition (and biological activity) of such solutions will change to some degree after withdrawal from the sea,^{*,**} for studies of the nature contemplated, ocean-derived solutions were deemed preferable to artificially compounded solutions.

Procedures were needed for collecting seawater under systematic and consistent environmental conditions and for storing it with minimum changes in composition. Since chemical and biological changes do

* F. W. Fink, Battelle Memorial Institute, Corrosion Research Division, personal communication.

**T. P. May, The International Nickel Co., Inc., Harbor Island Corrosion Laboratory, personal communication.

occur unavoidably during storage and subsequent use, analytical procedures were needed to monitor these changes for possible later correlation with release rate measurement data.

A literature survey was carried out and a method derived and tested for acceptable procurement, storage and analysis procedure for the seawater solutions to be used. Descriptions of the methods used are presented here in a form which may be used by other workers conducting similar experiments using seawater medium.

The collection, storage and analysis procedures selected for application to this work are generally well recognized and accepted methods. They are relatively easy to implement using ordinary laboratory equipment. However, some standard procedures have been modified to meet the specific requirements of the radionuclide release program. In several instances additional references for alternate analytical methods have been included. These describe promising new analytical techniques, primarily instrumental, that have not yet been extensively field-tested.

PROCEDURE FOR SEAWATER COLLECTION

The concentration of total salts in seawater varies with the location, depth, season, weather, and mixing conditions. However, the relative concentrations of most of the individual chemical components have been found to be nearly constant.^{3,17}

Since many of these compounds occur only in trace amounts, it is necessary to observe certain precautions when collecting samples so that trace concentrations are not altered by the act of collection. The collection scheme adopted for use in the radionuclide release program is not ideal; it has been compromised to a limited degree for convenience and economy of operation. Such compromises appear in the recommendation that surface waters (which are subject to more contamination and variation in chemical content) be obtained because of the ease of collection and the elimination of expensive sampling equipment. In addition, polyethylene sampling vessels are suggested as they are the most adaptable under the circumstances of collection. However, in the event the collection scheme is used in other investigations, it is noted that clear polyethylene containers are not generally recommended as a medium for sample collection when unaltered phosphate and nitrate are required.^{1,14} Both of these substances are adsorbed on polyethylene surfaces. Instead, amber pyrex vessels are preferred.

The collection procedure involves the procurement of unpolluted surface seawater samples by dip filling into polyethylene carboys. The detailed procedure for the water collection is included in Appendix I.

STORAGE OF SEAWATER

In conducting experiments simulating in situ conditions, the ideal situation would be to have a continuous supply of fresh seawater as the test medium. However, for the radionuclide release studies, fresh seawater can be procured only bi-weekly. In the interim, storage is necessary.

At present no satisfactory procedure has been developed for storing seawater without altering its corrosive properties.* Seawater is a complex liquid containing over a hundred different ions and populated by plant and animal life and their products. These can effect the deterioration of materials by chemical, electrochemical, and abrasive action.^{4,5} As mentioned previously, the use of artificial seawater is not feasible because its corrosion properties are different from those of natural seawater.

For the release rate experiments then, an attempt has been made to store natural seawater under such conditions as to control at least some of the known variables that cause the deterioration of the water. For convenience in handling, it is recommended that seawater be stored in large polyethylene carboys in the dark and at low temperatures (0-5°C). These conditions tend to inhibit the multiplication of bacteria,^{12,14} which in time would change the concentration of oxygen, phosphate, nitrate and perhaps other trace elements in the seawater. In addition, bacteria would introduce a considerable amount of waste and decay products. Large vessels are used to reduce the surface area-to-volume ratio and thus reduce the amount of nutrient and other elements and organisms adsorbed at the liquid-container interface.^{6,18}

A number of chemical preservatives have been suggested to prevent bacterial growth.^{7,14} However because of the possibility of interference, reaction, or other deleterious effects, their use is not recommended.

*T. P. May, *ibid.*

MONITORING ANALYSES OF SEAWATER

The following analyses for monitoring the chemical and biological composition of seawater are appropriate to the dissolution experimentation. In addition, generally they will serve for other investigations. However it should be recognized that other studies may require modification. Important criteria in choice of the specific methods noted are ease and economy in implementation and reliability of analyses.

Brief descriptions of the monitoring analyses are presented in the following paragraphs. The detailed procedures are described in Appendix II.

Microfiltration analysis. To determine the amount of particulate matter accumulated during the experiment. This gives an indication of biological growth during the period of an experiment and, correlated with other physical and chemical measurements, gives some useful information pertinent to the release rate studies.

Chlorinity analysis. To determine the total halogen content of the seawater. From this value the salinity or content of total dissolved solids per unit volume can be calculated. The chlorinity analysis is pertinent to the release studies since in the corrosion process - at least of metals - the chloride ion is probably the most corrosive constituent occurring in seawater in large quantities.⁴ Chlorinity analyses can serve another purpose: repetitive determinations give an indication of evaporative losses which may be incurred during the experimentation process.

Oxygen determination. This element is involved in the corrosion process of many materials - primarily by depolarizing cathodic areas, oxidation effects, and changing cell potentials. Changes in oxygen concentration also serve as an indicator of biological activity.^{7,15}

Alkalinity and pH. These parameters are important in the corrosion mechanism of many substances.¹⁰ Also they give an indication of

bicarbonate ion concentration, a corrosion-enhancing substance with respect to many materials.⁴

Phosphate and Nitrate determinations. Both of these are non-conservative substances, i.e., substances whose distributions are affected by biological processes. Evaluation of their uptake and regeneration acts as a rough index of biological activity.^{6,7,17}

APPENDIX I

SEAWATER COLLECTION PROCEDURE

1. Collect surface seawater at least two miles offshore - away from fresh water run-off or sewage outlets.

2. Just prior to the collection, rinse all containers with fresh seawater to reduce possible container-induced contamination.

3. Collect the water from either: (a) if weather permits, a small boat at least 100 yd forward of the ship (to prevent ship-induced contamination); or (b) in rough weather, the bow of the ship while it is moving at reduced speed.² In no instance use pumps or hoses.

4. Completely fill the 5-gallon polyethylene carboys to eliminate free space and cap tightly to prevent accidental spilling. An air-free system is needed to prevent a different equilibrium being established between the dissolved gases and the gases trapped in the airpocket.

5. Store the seawater samples in a cold, dark location. If refrigeration facilities are not available, keep the samples in a cool area and cover them with some opaque material such as a tarpaulin.

6. Keep a record of the collection and shipboard storage conditions. Weather and temperature conditions are needed to evaluate possible changes in the composition of the water due to dilution and aeration effects. Use the following log system:

- a. Date and time of collection.
- b. Location.
- c. Collection method used: moving ship; lowered boat; describe any other.

d. Weather conditions at time of sampling

- (1) Rainfall (approximate intensity)
- (2) Sea conditions
- (3) Water temperature
- (4) Air temperature

e. Weather conditions prior to sampling (estimated and reliability of estimate)

- (1) Rainfall
- (2) Sea conditions
- (3) Sample water temperature on return to land

f. Miscellaneous comments

- (1) Departure from standard collection procedure
- (2) Unusual conditions or difficulties encountered

APPENDIX II

MONITORING ANALYSES OF SEAWATER

MICROFILTRATION

The total particulate matter accumulated during the experiment is assayed by use of microfiltration-photographic techniques.

Apparatus and Equipment

35-mm camera.
Filtration apparatus with 5- μ membrane filters.
35-mm color film.

Procedure

Initially filter all seawater used in the experimentation process through a 5- μ membrane filter. At the conclusion of the experiment, refilter the water through a clean 5- μ filter. Estimate amount of particulate matter by visual examination of a photograph of the filter. Later a radiochemical and/or radioautographic assay can be made to obtain a quantitative estimate of the amount of activity retained by the particulate matter.

CHLORINITY* (and indirectly Salinity**) ^{1,16}

The concentration of total salts in the ocean is known to vary, depending on a number of factors including the location and depth.

* The number giving the chlorinity in grams per kilogram of a seawater sample is identical with the number giving the mass in grams of atomic weight silver just necessary to precipitate the halogens in 0.3285233 kg of the seawater sample.¹⁷

**Salinity is the total amount of solid material in grams contained in one kg of seawater when all carbonate has been converted to oxide, the bromine and iodine replaced by chlorine, and all organic matter completely oxidized.¹⁷

However, it is generally considered that the ratios of the concentrations of the major constituents are constant and independent of total salt concentration over a wide range.^{1,7,17} The analysis for a single constituent, then, will suffice in order to determine the total salts present, i.e. to determine salinity.

In the early twentieth century, the empirical relationships between chlorinity ($\text{Cl}^\circ/\text{oo}$) and Salinity (S°/oo) were established by Martin Knudsen.¹⁷ This relationship as adopted by an international commission in 1902 is

$$\text{S}^\circ/\text{oo} = 0.03 + 1.805 \text{Cl}^\circ/\text{oo}$$

In the method for the analytical determination presently used, equal volumes of a standard seawater (Eau de Mer Normal $\text{Cl}^\circ/\text{oo}$ 19.376) and of the sample are titrated with a silver nitrate solution to precipitate the halogens completely. The titration results then can be converted to chlorinity values (and salinity) by the use of Knudsen's Hydrographic Tables⁹ - a compilation relating the titration values of the standard seawater with an equal volume of sample to give the chlorinity of the sample.

Apparatus and Equipment

- Knudsen automatic buret.
- Knudsen automatic pipet.
- 200-ml beakers.
- Magnetic stirrer.
- Tygon tubing.

Reagents

Standard seawater (Eau de Mer Normal $\text{Cl}^\circ/\text{oo}$ = 19.376). Supplied by the Laboratoire Hydrographique, Charlottenlund Stot, Charlottenlund, Denmark.

Silver nitrate solution. Approximately 25 g of silver nitrate per liter of solution. Dilute (or concentrate) the solution so that, when titrated against standard seawater, the results fall within ± 0.150 of the standard chlorinity value (i.e., 19.376 ± 0.150). This is necessary so that the titration of subsequent seawater samples falls within the range of Knudsen's tables. Store the silver nitrate solution in a dark bottle.

Fluorescein indicator solution. Add 5 ml of 0.1 % solution of water soluble fluorescein powder to 100 ml of 1.0 % starch solution.

Procedure

Add 10 ml of seawater sample to a 200-ml beaker by means of the Knudsen pipet and then add 2 ml of the indicator solution. Titrate, using a Knudsen buret. Vigorously stir the contents of the beaker during the titration. As the endpoint is approached, rinse the sides of the beaker with distilled water.

Initially the seawater-indicator solution is yellow. At the end-point, however, the indicator is adsorbed on the silver chloride coloring the precipitate a light pink.

Calculation of Chlorinity

Calculate the chlorinity with the aid of Knudsen's Tables as follows:

Let N = the chlorinity of the standard seawater.

Cl = the unknown chlorinity of the seawater sample.

A = the buret reading for the titration of the standard seawater with $AgNO_3$ solution.

a = the buret reading for the seawater sample when titrated with $AgNO_3$.

Then $\alpha = N - A$

$k = Cl - a$, or $Cl = k + a$.

For each titration with standard seawater, determine α and from Knudsen's tables find the appropriate value of k for this value of α , and the titration value of the sample, a . The chlorinity is found by adding this value of k to the buret reading of the sample.

For example suppose $N = 19.376^{\circ}/_{\infty}$

$A = 19.302$

$a = 20.84$

$$N - A = \alpha = 19.376 - 19.302 = + 0.074$$

Then for $\alpha = + 0.074$ and a titration, $a = 20.84$, the value of k from the Tables is $+ 0.04$.

The required chlorinity is therefore

$$Cl^{\circ}/_{\infty} = k + a = + 0.04 + 20.84 = 20.88^{\circ}/_{\infty}$$

Notes.

1. The precision required for general work is $\pm 0.025^\circ/\text{oo}$.
2. The fluorescein indicator may be replaced by a potassium chromate (20 g/250 ml) solution. Three drops of this solution is sufficient for the titration. The endpoint is reached when the pale greenish yellow solution changes to a full yellow. A pale red is noted as the endpoint is exceeded.

Calculation of Salinity

The salinity may be found directly by use of Knudsen's tables or may be calculated from the chlorinity by the following relationship:

$$S^\circ/\text{oo} = 0.03 + (1.805 \text{ Cl}^\circ/\text{oo})$$

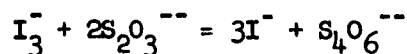
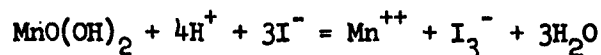
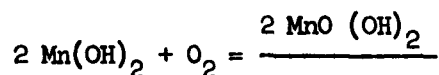
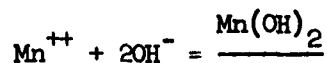
The method gives an accuracy of $\pm 0.02 \%$.

Alternate Methods

"Salinometers", Paquette, Robert, in Conference on Physical and Chemical Oceanography, NAS-NRC, publication 600 (1959).

OXYGEN DETERMINATION^{1,16}

A divalent manganese solution followed by strong alkali are added to the sample. The precipitated manganous hydroxide is converted by the dissolved oxygen present to basic hydroxides of higher valency states. When the solution is acidified in the presence of iodide, the oxidized manganese again reverts to the divalent state. Iodine, equivalent to the original dissolved oxygen content of the water, is liberated. This iodine is titrated with standardized thio-sulfate solution. The sequence of reactions is:



Apparatus and Equipment

60-ml amber polyethylene bottles
10- or 25-ml microburet
50-ml pipet
125-ml Erlenmeyer flasks

Reagents, Analytical Grade

Manganous sulfate solution. 450 g of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (Fe^{+++} free) is dissolved in distilled water to make a liter of solution.

Alkaline iodide solution. 500 g of NaOH and 150 g of KI are dissolved in distilled water and brought to one liter.

Sulfuric acid. Concentrated, 36 N.

Starch solution. A semi-preserved solution (stable for several months) is made as follows: Suspend 2 g of soluble starch in 300 to 400 ml of distilled water. Add an approximately 20 % sodium hydroxide solution with stirring until the solution becomes clear, and allow to stand for 1 to 2 hours. Neutralize with concentrated hydrochloric acid until the solution is just acid to litmus, and then add 2 ml of glacial acetic acid. Finally, dilute the solution to 1 liter with distilled water. Discard the indicator solution when the endpoint color is no longer a pure blue but takes on a green or brownish tint.

Sodium thiosulfate solution. Dissolve 3.5 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in distilled water and make up to 1 liter. Add a drop or two of carbon disulfide as a preservative and store in an amber bottle. This is about 0.015 N and should be checked at frequent intervals.

Potassium bi-iodate solution. Weigh out accurately about 0.3250 g of pure $\text{KH}(\text{IO}_3)_2$ (dried at 105°C for 1 hr). Dissolve in distilled water and make up to a liter. The strength of the solution is $w/32.5 = N$, where w is the weight in grams of the bi-iodate.

Standardization of the Sodium Thiosulfate Solution

Transfer 97 ml of seawater (or distilled water) to a 250-ml Erlenmeyer flask with stopper, and add 1 ml of concentrated sulfuric acid. Mix, add 1 ml of the alkaline-iodide reagent, and again mix. Add 10 ml of the standard potassium bi-iodate solution, swirl gently, stopper, and allow to stand 10 min in the dark. Titrate the liberated iodine with the thiosulfate to a pale yellow color. After adding 5 ml

of starch solution as indicator, complete the titration to the disappearance of the blue color.

A blank should be determined by repeating the process but omitting the bi-iodate. If more than 0.1 ml is required in the blank, ascertain the reagent responsible, and replace.

Procedure

Fill a 2-oz amber polyethylene bottle with seawater. Add 1 ml of the manganous sulfate solution from a pipet with its tip well below the surface of the water. Similarly add 1 ml of alkaline iodide solution. Stopper quickly, neglecting any overflow and taking care not to trap any air bubbles. Shake vigorously and, after the precipitate has partially settled, repeat the shaking. Allow the precipitate to settle as completely as possible. Introduce 1 ml of concentrated sulfuric acid and again shake until the precipitate dissolves and iodine is liberated. The subsequent titration should be made as soon as possible.

Transfer 50 ml to an Erlenmeyer flask, and immediately titrate the free iodine with the standard thiosulfate using the starch indicator (iodine is volatile; consequently the titration should be performed as quickly as possible). A 10- or 25-ml microburet should be used and read to at least the nearest 0.02 ml.

For a blank, titrate the seawater, reversing the order of reagent addition.

Calculations

When the entire contents of the bottle is used the oxygen content is given by

$$C = \frac{nN}{2(V-2)} \text{ mg atoms O}_2/1$$

$$C = \frac{nN}{4(V-2)} \times \frac{1000 \times 22400}{1000}$$
$$= \frac{nN}{V-2} \text{ ml O}_2 \text{ at STP/l}$$

where n = ml of thiosulfate
 N = normality of thiosulfate
 V = volume of bottle
 C = ml O₂ at STP/l

If less than the entire contents of the bottle is used

$$f = \frac{V - 2}{V} \times v$$

where

v = volume of seawater used in the titration and

C = $\frac{nN}{f} \frac{5600}{1}$ ml O₂ at STP/l

Alternate Method

D. D. Williams and R. R. Miller. An Instrument for On-stream Stripping and Gas Chromatographic Determination of Dissolved Gases in Liquids. Analytical Chemistry 34:657-59 (1962).

pH DETERMINATION^{1,16}

The pH of a sample is measured by a glass electrode and pH meter after taking certain precautions in sampling and standardization.

Apparatus and Equipment

Temperature-compensating pH meter.

50-ml, wide-mouth, screw cap polyethylene bottles, one per sample.

Reagents

Standard buffer, pH 7

Procedure

Measure the temperature of the buffer solution to the nearest 1°C, adjusting it, if necessary, so that the temperature lies between 20° and 25°. Set the temperature compensator of the pH meter to this temperature and standardize the instrument, at pH 7. Allow 5 min for the electrodes to reach equilibrium.

Adjust the temperature of the sample to within + 3°C of the temperature at which the pH meter was standardized. Measure the pH and temperature of the sample 3 to 5 min after immersing the electrodes. The solution should be gently swirled to assist the electrodes in reaching equilibrium. Note the pH and the temperature of the solution.

ALKALINITY DETERMINATION^{1, 16}

In seawater there is usually an excess of anions present which gives the water a slightly basic quality. Of the major anions, only the bicarbonate (HCO_3^-), carbonate (CO_3^{--}), and borate (H_2BO_3^-) ions are sufficiently strong bases to make a contribution to the basic character.

In the alkalinity determination, the endpoint is chosen so that these ions are combined with H^+ to give H_2CO_3 and H_3BO_3 . The results may be reported in milligram atoms per liter or, milliequivalents per liter of acid.

In practice 100 ml of seawater sample is mixed with 25 ml of 0.010 N hydrochloric acid (2.5 meq/l), and the pH of the resulting solution is measured. The standard acid in excess of that required to titrate the sample to the carbon dioxide endpoint is calculated from a knowledge of this pH and an empirical factor, f , the glass electrode hydrogen ion activity coefficient for varying chlorinities and pH's.

Apparatus and Equipment

pH meter.

200-ml, wide mouth, screw-cap polyethylene bottles, one per sample.

Reagents

Standard 0.010 N hydrochloric acid.

Standard buffer solution, pH 4.

Procedure

Pipet 25.00 ml of standard 0.010 N hydrochloric acid into a dry 200-ml polyethylene, wide-mouth, screw-cap bottle. Add from a pipet 100 ml of seawater. Stopper the bottle and mix the solution thoroughly. Warm the solution to room temperature and measure the pH exactly as described in the preceding method, except that the meter is standardized using a pH 4 buffer solution.

Calculations

alkalinity = total amount of acid added - excess acid.

The total amount of acid added per liter of sample is (in milliequivalents).

$$\frac{1000}{\text{vol of sample (ml)}} \times (\text{ml acid added}) (\text{Normality acid}) \quad (1)$$

The amount of acid neutralized (in milliequivalents) is

$$\frac{1000}{\text{vol of sample (ml)}} \times (\text{vol of sample (ml)} + \text{ml HCl added})(C_H) \quad (2)$$

where C_H is the excess acid concentration.

Since $\text{pH} = -\log C_H \cdot f$

where f takes into account the ionic strength of the solution

and if $a_H = 10^{-\text{pH}}$

then $C_H = a_H/f$

The final form of the equation (from 1 and 2 then is

$$2.5 - 1250a_H/f = \text{alkalinity}$$

a_H and f may be obtained from Tables I and II.

Notes

1. For chlorinity values between 12°/oo and 18°/oo and final pH values between 3.0 and 3.9, the total alkalinity may be read directly from Table III without incurring appreciable errors.

2. If the final pH is greater than 4.0, remove the electrodes, but do not wash them and pipet 5 ml of 0.01 N acid into the bottle. Mix the solution and again measure the pH. Calculate a_H and f as above and substitute in the equation: $\text{alkalinity} = 3.00 - (1300a_H/f)$.

PHOSPHATE DETERMINATION¹⁶

The estimation of orthophosphate depends upon the formation of a phosphomolybdenum complex and its reduction to molybdenum blue. A correct physicochemical formulation of the reaction is still not available. However, it is very sensitive to changes in reagent concentration and therefore it is essential to adhere strictly to empirically determined optimum conditions.

Apparatus and Equipment

Spectrophotometer, UV and visible

TABLE I^a

Conversion of pH to Hydrogen Ion Activity From the Relation $a_H = 10^{-\text{pH}}$

For a pH of $Q + v$ (where v is the decimal part) find N from the Table in terms of v and substitute in the equation:

$$a_H = N \times 10^{-Q}$$

v	N	v	N	v	N
0.00	1.000	0.34	0.457	0.67	0.214
0.01	0.977	0.35	0.447	0.68	0.209
0.02	0.955	0.36	0.437	0.69	0.204
0.03	0.933	0.37	0.427	0.70	0.200
0.04	0.912	0.38	0.417	0.71	0.195
0.05	0.891	0.39	0.407	0.72	0.191
0.06	0.871	0.40	0.398	0.73	0.186
0.07	0.851	0.41	0.389	0.74	0.182
0.08	0.832	0.42	0.380	0.75	0.178
0.09	0.813	0.43	0.372	0.76	0.174
0.10	0.794	0.44	0.363	0.77	0.170
0.11	0.776	0.45	0.355	0.78	0.166
0.12	0.759	0.46	0.347	0.79	0.162
0.13	0.741	0.47	0.339	0.80	0.158
0.14	0.725	0.48	0.331	0.81	0.155
0.15	0.709	0.49	0.324	0.82	0.151
0.16	0.692	0.50	0.316	0.83	0.148
0.17	0.676	0.51	0.309	0.84	0.144
0.18	0.661	0.52	0.302	0.85	0.141
0.19	0.646	0.53	0.295	0.86	0.138
0.20	0.631	0.54	0.288	0.87	0.135
0.21	0.617	0.55	0.282	0.88	0.132
0.22	0.603	0.56	0.275	0.89	0.129
0.23	0.589	0.57	0.269	0.90	0.126
0.24	0.575	0.58	0.263	0.91	0.123
0.25	0.562	0.59	0.257	0.92	0.120
0.26	0.549	0.60	0.251	0.93	0.117
0.27	0.537	0.61	0.245	0.94	0.115
0.28	0.525	0.62	0.240	0.95	0.112
0.29	0.513	0.63	0.234	0.96	0.110
0.30	0.501	0.64	0.229	0.97	0.107
0.31	0.490	0.65	0.224	0.98	0.105
0.32	0.479	0.66	0.219	0.99	0.102
0.33	0.468				

a. From reference 16, p. 178 (reproduced courtesy of J.D. Strickland Fisheries Research Board of Canada).

TABLE II^a

Factors for Total Alkalinity Measurement

Factor f in equation:
 Total alkalinity = $2.500 - 1250 \text{ ap}/f$
 as a function of chlorinity or salinity

pH Range	Cl ^o /oo = 2	4	6	8	10	12-18	20
	S ^o /oo = 3.5	7	11	14.5	18	21-33	36
	f	f	f	f	f	f	f
2.8-2.9	0.865	0.800	0.785	0.775	0.770	0.768	0.773
3.0-3.9	0.845	0.782	0.770	0.760	0.755	0.753	0.758
4.0	0.890	0.822	0.810	0.800	0.795	0.793	0.798

TABLE III^a

Total Alkalinity Calculation

Total alkalinity (1 litre 20°C) as a function of the final pH of the solution obtained from 100.0 ml of sample and 25.00 ml of 0.01000 N hydrochloric acid. This Table may be used for samples of chlorinity between 12^o/oo and 18^o/oo, or salinities between 22^o/oo and 33^o/oo.

pH	Total Alkalinity	pH	Total Alkalinity	pH	Total Alkalinity
3.00	0.84	3.30	1.67	3.60	2.08
3.02	0.92	3.32	1.71	3.62	2.10
3.04	0.99	3.34	1.74	3.64	2.12
3.06	1.06	3.36	1.77	3.66	2.14
3.08	1.12	3.38	1.81	3.68	2.15
3.10	1.19	3.40	1.84	3.70	2.17
3.12	1.24	3.42	1.87	3.72	2.18
3.14	1.30	3.44	1.90	3.74	2.20
3.16	1.35	3.46	1.93	3.76	2.21
3.18	1.40	3.48	1.95	3.78	2.23
3.20	1.45	3.50	1.98	3.80	2.24
3.22	1.50	3.52	2.00	3.82	2.25
3.24	1.55	3.54	2.02	3.84	2.26
3.26	1.59	3.56	2.04	3.86	2.27
3.28	1.63	3.58	2.06	3.88	2.28
				3.90	2.29

a. From reference 16, pg. 179 (reproduced courtesy J. D. Strickland).

A pair of 5-cm silica cells
2-Oz, amber polyethylene, screw-cap bottles (1 per sample)

Reagents, Analytical Grade

Ammonium molybdate tetrahydrate $((\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O})$ solution.

Add 20 g of ammonium molybdate (dissolved in 200 ml of distilled water) to 600 ml of 50 % by volume sulfuric acid. Store in an amber polyethylene bottle. Discard when a definite blue coloration develops.

Stannous chloride solution. Stock: dissolve 4.3 g of stannous chloride dihydrate $(\text{SnCl}_2 \cdot 2\text{H}_2\text{O})$ in 20 ml of concentrated hydrochloric acid and dilute to 100 ml with distilled water. The solution should be kept over a piece of mossy tin and stored in a polyethylene bottle. It should be renewed at least every 3 months. Prior to use, dilute 5 ml of the stock solution to 50 ml using a 5 % hydrochloric acid solution in distilled water. The diluted solution should be prepared daily or stored for short periods under mineral oil.

Standard phosphate solution. Make a standard solution of anhydrous potassium dihydrogen phosphate, KH_2PO_4 (0.816 g/liter, 1 ml = 6 μg atom P). Store in a dark bottle with 1 ml of chloroform. The solution is stable for several months.

Synthetic seawater. Dissolve 25 g of sodium chloride and 8 g of magnesium sulfate heptahydrate $(\text{MgSO}_4 \cdot 7\text{H}_2\text{O})$ per liter of distilled water and store in a polyethylene bottle. The phosphorous blank extinction on this solution should not exceed 0.25.

Calibration. Dilute 5.0 ml of the standard phosphate (6 μg atom P/ml) solution to 500 ml with synthetic seawater. Store in a dark bottle with 0.5 ml of chloroform and renew every 10 days.

$$1 \text{ ml} = 6.0 \times 10^{-2} \mu\text{g atom P}$$

Prepare a standard solution of 5 ml of the dilute phosphate solution made to a volume of 100 ml with synthetic seawater.

$$1 \text{ ml} = 3 \times 10^{-3} \mu\text{g atom P}$$

or

$$1 \text{ liter} = 3.0 \text{ gram atom}$$

Transfer 50 ml of the solution to a dry polyethylene bottle and at the same time prepare 50 ml of synthetic seawater as a blank. Carry out the phosphate determination as described below. Calculate the factor F from the expression

$$F = \frac{3.00}{E_s - E_b}$$

Where E_s is the extinction of the standard and E_b is the mean extinction of the blank. The value for F should be between 4 and 4.5 for salinities above 20 ‰ (for salinities less than 20 ‰ the synthetic seawater should be diluted to the salinity of the natural seawater).

Procedure

Warm the sample (50 ml) to a temperature between 18 and 23°C (both the sample and standard should be analyzed at the same temperature) and add 0.5 ml of molybdate solution.

Transfer the solution to a 5-cm cell that has been rinsed with distilled water (the cell should not be rinsed with sample). Three minutes after molybdate addition, measure the extinction against a distilled water blank at a wavelength of 700 mμ. Record the extinction E_m to the nearest 0.001 unit.

Pour the solution back into the vessel containing the remainder of the sample and molybdate. Drain the cell but do not wash it. Add 0.5 ml of dilute stannous chloride and mix. Refill the 5-cm cell with the reduced solution, rinsing once. Exactly 7 min after adding the stannous chloride, re-measure the extinction again against a distilled water blank. Let this extinction be E_r . Subtract the reading E_m from the reading E_r to give $(E_r - E_m)_{\text{sample}}$.

Obtain a reagent blank by carrying out the determination exactly as described for the sample but using 50 ml of distilled water in its place. Evaluate the expression $(E_r - E_m)_{\text{distilled}}$.

Calculate the phosphate concentration in microgram-atoms of phosphate per liter from the expression

$$\mu\text{g at P/liter} = ((E_r - E_m)_{\text{sample}} - (E_r - E_m)_{\text{distilled}}) \times F$$

where F = the same factor as used in the calibration data.

NITRATE DETERMINATION*

The method depends on the reduction of nitrate to nitrite by means of hydrazine (catalyzed by copper) in a solution buffered at pH 9.6. After the reduction is completed acetone is added to complex any residual hydrazine. The nitrite that is produced is diazotized with sulfanilimide and coupled with 1-Naphthylamine to form a colored azo dye whose extinction is measured.

*Method of Mullin and Riley, modification by Higaki.⁸

Salt error corrections, which take into account the efficiency of the reduction procedure in salt water as compared to distilled water, are applied. The unknown nitrate concentration is then obtained by comparison with a previously constructed calibration curve.

Apparatus and Equipment

Spectrophotometer, UV and visible.
A pair of silica cells, either 1-cm or 5-cm.
50-ml volumetric flasks, one per determination.

Reagents

Phenol-sodium phenate buffer solution. Stock: Dissolve 9.40 g of phenol (C_6H_5OH) in distilled water to make 250 ml of solution. Filter through a sintered glass filter of porosity 4.

For use: pipet 50 ml of the stock solution into a 100-ml graduated flask, add 16 ml of 1.0 N sodium hydroxide solution, dilute to volume, and mix thoroughly. Store in an amber glass bottle and reject when it becomes discolored.

Copper sulfate catalyst. Dissolve 0.0393 g of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in distilled water to make 100 ml of solution.

Hydrazine sulfate solution. Prepare a solution containing 1.20 g of hydrazine sulfate (N_2H_4) \cdot H_2SO_4 in 250 ml of distilled water. Filter through a sintered glass filter of porosity 4.

Hydrazine-copper reducing agent. Mix 25 ml of hydrazine sulfate solution with 5 ml of copper sulfate catalyst and diluted to 50 ml. Prepare this solution daily.

Sulfanilic acid solution. Add 12.9 ml of concentrated hydrochloric acid to a solution of 0.30 g of sulfanilic acid ($NH_2C_6H_4SO_3H \cdot H_2O$) in 80 ml of water. Dilute to 100 ml.

1-Naphthylamine solution. Dissolve 0.060 g of recrystallized 1-Naphthylamine hydrochloride ($C_{10}H_7NH_2 \cdot HCl$) in 80 ml of water containing 1 ml of concentrated hydrochloric acid. Dilute to 100 ml.

Sodium acetate solution. Prepare a 2 M solution by dissolving 27.2 g of hydrated sodium acetate per 100 ml.

Acetone

Standard nitrate solution (10 $\mu\text{g NO}_3\text{-N/ml}$). 0.0722 g of potassium nitrate per liter. Dilute as required.

Procedure

Place 40 ml of the seawater sample (freshly filtered through a Whatman # 42 filter paper) in a 50-ml graduated flask, and add 2 ml of phenol-sodium phenate buffer while swirling the solution. Add 1 ml of the hydrazine copper reagent, mix and set aside in the dark.

Allow the reaction to continue for 24 hours. Add 2 ml of acetone, then 2 ml of sulfanilic acid reagent with shaking. After not less than 5 min, add 1 ml of the 1-Naphthylamine reagent, shake and add 1 ml of 2 M sodium acetate. Dilute to 50 ml and mix thoroughly. After 15 min measure the optical density of the solution at 524 m μ in a cell of appropriate length.

Calibration and Determination of Salt Error

Calibration. Prepare a distilled water calibration curve by analyzing solutions of known added nitrate concentration (in the range of 0-50 $\mu\text{g NO}_3\text{/liter}$). Use distilled water without nitrate as a blank.

Similarly, prepare a seawater calibration curve. Use seawater without nitrate as a blank.

Salt error factor. The salt error, defined as the ratio of the optical density from the nitrite formed in seawater to that formed from the same amount of nitrate in distilled water, is calculated from the calibration curves.

The salt error is a function of salinity and perhaps one or more other variables.⁸ It should be occasionally checked by adding a known amount of nitrate to a single sample of seawater. After subtraction of the blank, fit the resulting value to the previously obtained seawater calibration curve. If the curves do not coincide, recalculate the salt error factor from freshly prepared distilled water and seawater calibration curves.

Calculations

Analyze 40 ml of sample seawater according to the method as outlined above. Multiply the blank obtained by conducting the reduction

procedure on 40 ml of distilled water by the salt error factor. Subtract this amount from the optical density reading of the sample seawater.

This value is divided by the salt error correction factor and the result is fitted into the distilled water calibration curve to determine the nitrate concentration.

Example

Assuming the salt error factor to be 0.667

Reduction procedure on 40 ml seawater sample	0.036
Reduction procedure on 40 ml alkali distilled water (blank)	0.030
Nitrate in reagent (blank) corrected for salt error 0.030×0.677	= 0.020
Optical density due to nitrate (1) - (3)	= 0.016
Corrected for salt error $0.016 \div 0.667$	= 0.024

Determine the nitrate concentration from the distilled water calibration curve.

The same result can be obtained by multiplying the optical density of the blank by the salt error factor and subtracting the result from the optical density of the sample. This value is then placed into the salt water calibration curve and the concentration of the nitrate determined.

Alternate Method

T. J. Chow and M. S. Johnstone. Determination of Nitrate in Seawater. Anal. Chim. Acta 27:41-46 (1962).

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was devised for the collection, storage and analysis of the water. In brief, the method stipulates: (1) off-shore collection of surface water in polyethylene carboys which are subsequently stored, at a reduced temperature, in a dark location and (2) monitoring biological and chemical composition via micro-filtration, chlorinity, oxygen, alkalinity, phosphate and nitrate analyses.

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